AD- 2791

UNCLASSIFIED

ARMY MEDICAL RESEARCH LABORATORY

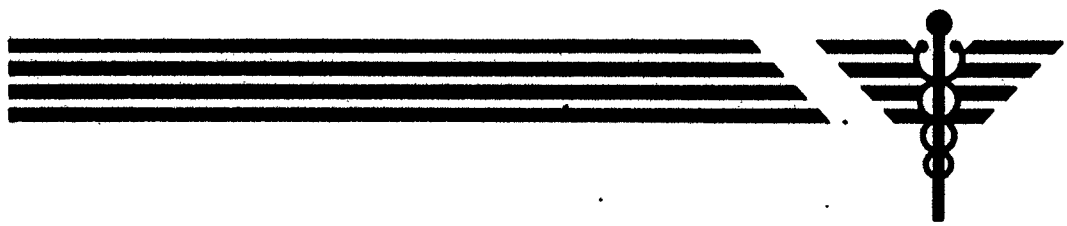
FORT KNOX, KENTUCKY

REPORT NO. 103

17 November 1952

PROTEIN COMPOSITION AND AZORUBIN-BINDING CAPACITY IN SERUM OF RABBITS SUBJECTED TO TOURNIQUET SHOCK*

*Subtask under Environmental Physiology, AMRL Project No. 6-64 12-028, Subtask, Effect of Stress on Physicochemical Behavior of Blood Proteins.



MEDICAL RESEARCH AND DEVELOPMENT BOARD
OFFICE OF THE SURGEON GENERAL
DEPARTMENT OF THE ARMY

REPORT NO. 103

PROTEIN COMPOSITION AND AZORUBIN-BINDING CAPACITY IN SERUM OF RABBITS SUBJECTED TO TOURNIQUET SHOCK*

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Report No. 103 17 November 1952
Project No. 6-64-12-028
Subtask AMRL S-10
MEDEA

ABSTRACT

PROTEIN COMPOSITION AND AZORUBIN-BINDING CAPACITY IN SERUM OF RABBITS SUBJECTED TO TOURNIQUET SHOCK

OBJECT

In order to characterize certain metabolic alterations, the protein composition of the serum and the azorubin-binding capacity (ABC) of the serum albumin were studied in rabbits subjected to tourniquet shock. Abnormalities in the ABC values are expected to assist in elucidating certain phases of metabolic deviations observed in traumatic shock.

RESULTS

A procedure was developed to produce reproducible tourniquet shock in rabbits. The concentrations of total protein and albumin were found to be decreased markedly in the sera of rabbits subjected to tourniquet shock. Non-protein nitrogen was elevated. The electrophoretic analysis demonstrated an increase in the alpha globulin components. The ABC values were decreased in the "shocked" rabbits.

CONCLUSIONS

Use of the rabbit for shock experiments may be of advantage when larger amounts of blood are needed, or when periodic sampling is desirable. The metabolic alterations occurring in tourniquet shock apparently have a specific influence on the concentrations of the different protein components of the rabbit serum. A decreased azorubin-binding capacity of serum albumin is believed to be a general phenomenon in shock conditions.

RECOMMENDATIONS

Using the rabbit as an experimental animal, the mechanism of the lowering of the azorubin-binding capacity should be studied further.

This is expected to result in a better understanding of metabolic alterations in shock as well as in other conditions causing abnormal ABC values.

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PROTEIN COMPOSITION AND AZORUBIN-BINDING CAPACITY IN SERUM OF RABBITS SUBJECTED TO TOURNIQUET SHOCK

I. INTRODUCTION

During the various phases of traumatic shock, certain alterations in the protein metabolism take place which may be reflected in the composition of the serum (1). Studies in rats subjected to tourniquet shock failed to reveal marked changes in the concentrations of the various serum protein components (2). It was considered to be of interest, therefore, to investigate the influence of tourniquet shock on the serum protein pattern in rabbits.

Use of the rabbit in biochemical studies on shock may be of particular advantage when larger amounts of blood are needed, or when periodic sampling is desired. One would avoid the pooling of samples obtained from several animals as was necessary in shock experiments on rats (2). The development of a standardized procedure to produce tourniquet shock in the rabbit was, therefore, attempted.

The azorubin-binding capacity (ABC) of serum albumin has been found to be decreased in rats subjected to tourniquet shock (2). It was investigated whether a similar lowering of the ABC could be demonstrated in the "shocked" rabbit. The result would indicate whether a reduction of the ABC values could be considered a general phenomenon in traumatic shock, and possibly would offer more information for evaluating the metabolic alterations which result in decreased ABC values. Recognition of the mechanism by which the ABC of serum albumin is lowered in vivo might result in a better understanding of the pathological conditions under which decreased ABC values have been observed in several species (2, 3).

II. EXPERIMENTAL PROCEDURES

A. Chemical Procedures

Total protein and non-protein nitrogen were determined as described in a previous report (2). In a number of normal sera (Table 2) the albumin values were obtained by the sodium sulfate fractionation method (26.8%) as outlined before (2). In normal sera, this procedure gave albumin figures similar to those estimated by electrophoretic analysis in Michaelis buffer of pH 8.0 (Table 1,

Rabbit No. 1 and 2). Comparative albumin determinations in the sera of rabbits subjected to various degrees of tourniquet shock, however, showed less conformity (Table 1, Rabbit No. 3-11).

TABLE 1

COMPARISON OF ALBUMIN CONCENTRATIONS OF RABBIT SERA*, DETERMINED BY CHEMICAL AND ELECTROPHORETIC ANALYSIS

Rabbit No.-	1	2	3	4	5	6	7	8	9	10	11
Total Protein gm%	5.69	5.55	4.74	5.35	6.09	4.97	4.66	4.99	4.89	5.24	6.52
NPN mg%	48.5	44.5	52.8	53.5	63.0	49.5	46.8	52.5	54.5	70.8	86.0
Albumin gm% (Na_2SO_4 precipitation)	4.29	4.08	2.85	3.69	3.98	2.90	2.71	3.05	3.19	3.79	3.75
Albumin gm% (electrophoresis)	4.23	4.10	3.25	3.58	3.95	3.26	2.81	3.30	2.91	3.85	4.39

*Rabbits No. 1 and 2 normal; rabbits No. 3-11 subjected to various degrees of tourniquet shock.

B. Electrophoretic Analysis

The electrophoretic albumin values, used for a comparison with the figures obtained by sodium sulfate fractionation (Table 1), were determined as described previously (2), except for the application of a Michaelis buffer of pH 8.0, $\mu = 0.1$. The compact Tiselius apparatus* was employed in these experiments.

All other electrophoretic studies were done in a standard size electrophoresis apparatus** using the long analytical cell. The veronal-sodium acetate-chloride (Michaelis) buffer, pH 8.6, $\mu = 0.1$, was employed. Comparative electrophoretic analyses of a normal rabbit's serum, in veronal buffer, pH 8.60; sodium bicarbonate, pH 8.68; and Michaelis buffer, pH 8.60, gave similar albumin values (58.0%; 55.0%; and 56.1%, respectively). The samples were dialyzed for 22 hours at 2°C in a mechanically stirred dialyzer*** (4), the buffer being changed six times. The electrophoretic separations were run for 150 minutes at about 8 volts per cm. The conductivity was measured**** in a Shedlovsky cell at the temperature of the water bath (+0.4°C).

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* Model 38 of the Perkin-Elmer Corporation, Glenbrook, Connecticut

** Frank Pearson Associates, New York 12, New York

*** American Instrument Company, Inc., Silver Spring, Maryland

**** Conductivity bridge built by Dember Precision for Frank Pearson Associates.

The electrophoretic patterns obtained in the compact apparatus were evaluated as described previously (2). The same procedure was employed to estimate the albumin concentrations of the sera analyzed in the standard size electrophoresis apparatus, using the descending boundaries. These albumin values are given for all sera reported in Tables6 and 7. The globulin components of these sera were determined in the following way.

On the tracings of the descending boundaries, at 3 times linear enlargement, 6 globulin components were apparent in most cases (Figure 1, A and B). The total globulin area, therefore, was divided accordingly by drawing vertical lines from the lowest point between two adjacent peaks (5) and the six components designated as alpha$_1$, alpha$_2$, beta$_1$, beta$_2$, beta$_3$, and gamma globulins. Of the 72 possible dividing lines between the protein components of the 12 sera reported in Tables 6 and 7, nine could not be clearly defined. The locations of these indistinct separations, observed between the components of alpha globulin and beta globulin, are marked by an asterisk in Table 7. To obtain these nine dividing lines by interpolation, all other dividing lines were first expressed in per cent of total migration of the corresponding albumin peaks. These values for the different globulin components were averaged separately for the sera of the normal and "shocked" rabbits. The average figures thus obtained were used as substitutes in the nine cases where the dividing lines were not clearly defined on the patterns, and recalculated for the original migration (distance between starting line and albumin peak) of the individual sera. The recalculated values indicated where the dividing lines had to be drawn on the original tracings. This procedure of interpolation is believed to permit a reasonable use of the electrophoretic data of all sera for a computation of the six globulin components according to migration rates. It can be applied only if the data of a group of similar sera are available.

The electrophoretic analysis of a mixture of rabbit serum and azorubin was performed as described previously for rat serum (2). Michaelis buffers of pH 8.6, 7.2 and 6.2 $\mu = 0.1$, were used. The results were recorded on color film.

C. Determination of ABC

The ABC values were determined as outlined previously (6). The albumin concentrations used for the calculation of the specific ABC were determined by chemical fractionation for the normal sera reported in Table 2, and in all other cases by electrophoretic analysis.

In order to obtain information on possible in vivo variations of the ABC values of individual animals over a certain period of time, the sera of 10 normal rabbits were analyzed for total protein, albumin (sodium sulfate fractionation) and specific ABC, and the analyses were repeated after a period of (in general) about 4 weeks. Table 2 shows that the specific ABC values estimated in sera obtained at different times were practically identical. An exception was rabbit No. 59, the specific ABC of which increased by almost 23%. It should be mentioned, however, that this animal was the only one of the group to show weight loss and considerable reduction of the serum albumin concentration at the time of the second analysis.

TABLE 2

REPRODUCIBILITY OF SPECIFIC ABC VALUES
IN SERA OF NORMAL RABBITS
All Protein Values in Duplicate

Rabbit No.	Date of Exp.	Weight kg	Total Protein gm%	Albumin gm%	Globulin gm%	Specific ABC in 10^{-5} Moles Azorubin/Gram Albumin
51	11-17	2.22	5.66	4.16	1.50	2.19
	12-18	2.78	5.73	4.24	1.49	2.21
52	11-17	1.82	5.95	4.24	1.71	2.18
	12-18	2.04	5.86	4.24	1.62	2.27
53	11-20	2.36	5.81	4.18	1.63	2.22
	12-18	2.77	6.05	4.31	1.74	2.18
54	11-20	2.81	5.93	4.20	1.73	2.27
	12-19	3.38	6.13	4.35	1.78	2.27
55	11-21	2.74	5.92	4.59	1.33	2.21
	12-19	3.18	6.05	4.53	1.52	2.16
56	11-21	2.21	6.21	4.40	1.81	2.02
	12-19	2.99	6.11	4.29	1.82	2.01
57	11-22	2.33	6.14	4.17	1.97	2.26
	12-20	2.61	5.92	3.86	2.06	2.38
58	11-22	2.54	6.44	4.97	1.47	2.00
	12-20	3.12	6.40	4.48*	1.92	2.25
59	12-5	2.65	6.14	4.37	1.77	2.08
	12-20	2.64	5.65	3.57	2.08	2.55
60	12-5	2.50	5.92	3.55	2.37	2.26
	12-20	2.55	5.70	3.61	2.09	2.22

*Duplicate value was lost. This figure represents one determination.

The chromatographic analyses were done at a temperature between 23° and 26°C. In all cases shown in Table 6, the ABC values of the normal and "shocked" sera designated by the same "Exp. No."

were determined simultaneously. Accordingly, the calculation (7) of the "decrease of specific ABC" (Table 6, column 10) was based on a comparison of the values obtained in these pairs of sera.

D. Animal Experiments

New Zealand Hybrid male rabbits fed on Purina rabbit chow checkers were used for all studies. The temperature of the animal room was kept at about 24° ± 2°C. The rabbits were left without food and water for 8 hours before and throughout the experiment; in some preliminary studies, the pre-experimental fasting period was 24 hours. Weights given are those of the non-fasted animals.

For the experiments summarized in Table 2, the blood was obtained by puncturing the ear veins. All other sera were prepared from blood drawn from the heart. The syringes were coated with paraffin oil as described previously (2).

Production of tourniquet shock in rabbits by occluding one or both hind legs has been described by several authors (8, 9, 10). In the present studies, an attempt was made to occlude both hind legs for varying periods of time. Several sizes of rubber bands, rubber tubing, and different types of wire were placed as high as possible on the thighs and fastened by various means. The preliminary experiments did not produce satisfactory results, the difficulty being mainly that the tourniquets did not stay in place, although the hair was removed from the area of application. The degree of shock obtained was not uniform. A series of experiments in which the front legs of the rabbits were occluded finally led to the adoption of the following procedure.

The hair was removed high up on both front legs and the clipped area treated with merthiolate (1:1000). After 2 to 2-1/2 hours, elastic rubber bands (Eberhard Faber No. 64) were applied in seven tight turns, as high as possible, and left on for 16 hours. The blood was drawn 5 hours after removal of the tourniquets. In a series of 77 rabbits, the survival times after release of the rubber bands were observed without further experimentation.

E. Hematocrit

The blood was drawn into heparinized syringes (0.1 mg heparin per 2-ml syringe) and the hematocrit values determined in Wintrobe tubes (11) by centrifuging for 30 minutes at 2200 g (3500 rpm). The

reproducibility of duplicate runs of the same samples was determined. Calculation of the deviations for the duplicates gave a standard error of ± 0.23% with a fiducial limit of ± 0.71%*.

III. RESULTS AND DISCUSSION

The mortality data summarized in Table 3 show that 87% of the rabbits subjected to tourniquet shock died within 24 hours after removal of the rubber bands. Using mortality as a criterion, the severity of shock produced by the present procedure therefore lies between that obtained at 24°C by Katzin, Ricca and Warren (9) and that observed by Canzanelli, Guild and Rapport (8). More than half of the animals died within a period of 8 hours after release of the tourniquets.

TABLE 3

MORTALITY RATE OF RABBITS SUBJECTED TO TOURNIQUET SHOCK

Studies on 77 Animals; Average Weight 2.07 Kg.*

Hours After Removal of Tourniquets	No. of Animals Dead Within Time Periods	Avg. WT. Kg	% Mortality	% Cumulative Mortality
0-1	2	2.18	2.6	2.6
1-2	9	2.00	11.7	14.3
2-4	23	2.04	29.9	44.2
4-8	16	2.13	20.8	65.0
8-24	17	2.04	22.1	87.0
24-48	1	2.22	1.3	88.3
48-72	0	--	0	88.3
After 72 Hours	9 Alive	2.13	0	88.3

*S.E. = ± 0.024; F.L. = ± 0.06

* The fiducial limits (F. L.) have been calculated in this paper for a probability level of 0. 01 (= 0. 99 fiducial interval). See reference (12).

The blood was obtained in general about 5 hours after termination of the tourniquet period. It follows from the mortality data given in Table 3 that a severe state of shock was produced under such conditions. Table 4 summarizes the total protein, non-protein nitrogen and hematocrit values.

TABLE 4

HEMATOCRIT, TOTAL PROTEIN, AND NPN
IN NORMAL AND 'SHOCKED' RABBITS

	Weight kg	Hematocrit %	Total Protein gm%	NPN mg%
A. Normal Rabbits				
Average Values	2.38	45.4	6.03	41.4
Standard Error±	0.057	0.65	0.072	1.01
Fiducial Limits*±	0.15	1.8	0.20	2.7
No. of Sera Analyzed for Average Values	59**	27	32	40
B. Rabbits Subjected to Tourniquet Shock				
Average Values	2.26	47.2	4.99	62.6
Standard Error±	0.038	1.26	0.140	2.48
Fiducial Limits* ±	0.10	3.5	0.40	6.8
Significant Deviation from Normal Group, in %	--	--	-17.3	+ 51.2
No. of Sera Analyzed for Average Values	39**	28●	20	33●

*Probability level 0.01

**Total number of rabbits used in these experiments.

● In 3 rabbits of this series, the blood was drawn about 2 hours after removal of the tourniquets. The values obtained in these 3 animals were well within the range of the series.

The hematocrit values observed in the blood of the rabbits subjected to tourniquet shock did not show significant differences from normal, suggesting that neither hemoconcentration nor hemodilution had occurred. The range of hematocrit distribution is given in Table 5. These results are similar to those obtained by Cole and associates (13), as well as by Nastuk (14) who could not demonstrate significant changes in the hematocrit values of rabbits as a result of gravity shock. Gray, Botkin, Moulden and Jensen (15) observed increased hematocrit values in rats subjected to a tourniquet shock procedure which produced about the same 24 hour mortality (89.5%) as the present method.

TABLE 5
DISTRIBUTION OF HEMATOCRIT VALUES IN NORMAL AND 'SHOCKED' RABBITS

Average Value for Normal Rabbits = 45.4*
Average Value for 'Shocked' Rabbits = 47.2**

Hematocrit Values Within	38-42	42-46	46-50	50-54	54-58	58-62
No. of Normal Rabbits	4	12	9	2	0	0
Average Weight Kg	2.05	2.11	2.18	1.66	-	-
No. of 'Shocked' Rabbits	5	9	6	2	3	3
Average Weight-Kg	2.29	2.26	2.23	2.23	2.14	2.14

*S.E. = ± 0.65; F.L. = ± 1.8
**S.E. = ± 1.26; F.L. = ± 3.5

The total protein concentration in the sera of the "shocked" rabbits was significantly lower than that obtained in the sera of normal animals (Table 4). This is in accordance with similar observations in other species (1). The non-protein nitrogen (NPN) was found to be significantly elevated, which also is a general finding in traumatic shock (1). Cole and associates (13) observed a marked increase of the NPN values in the serum of rabbits subjected to gravity shock. They found, however, a slight elevation in the total protein concentration. Cursory inspection of the present data showed the lack of characteristic correlation between hematocrit, total protein and NPN values obtained in the samples of the individual animals (Table 4). Three out of the 33 sera of "shocked" rabbits analyzed had NPN values below the upper limit (44.1) calculated for normal sera (Table 4). This is in accordance with the expectation from the mortality data given in Table 3.

The results of a more detailed analysis of the sera of 6 each of the normal and "shocked" rabbits are given in Tables 6 and 7. The low total protein concentration observed in the sera of the animals with tourniquet shock (Table 6, B) could be accounted for mainly by

a decrease in the absolute albumin concentration. The relative albumin concentration, estimated as per cent of total protein, was also reduced. These albumin values were determined by electrophoresis. Preference of this method was based on a comparison between the chemical and the electrophoretic estimation of albumin in the sera of normal and "shocked" rabbits, details of which were given in the Experimental Procedures and Table 1. The average absolute globulin concentration was found to be lowered in the "shocked" rabbits. The deviation from normal, however, was much less pronounced and less uniform than in the case of albumin.

TABLE 6

SERUM COMPONENTS AND AZORUBIN-BINDING CAPACITY OF ALBUMIN IN NORMAL AND 'SHOCKED' RABBITS

1	2	3	4	5	6	7	8	9	10
Exp. No.	Rabbit No.	Wt. kg	NPN mg%	Protein gm%	Albumin by Electrophoresis % of Total Protein	Albumin* gm%	Globulin** gm%	Specific ABC in 10^{-5} Moles Azorubin per Gram Albumin	%Decrease of Specific ABC ***
A. Normal Rabbits									
1	149	2.30	44.5	5.58	58.8	3.28	2.30	2.00	
2	153	2.44	40.5	5.84	63.2	3.69	2.15	2.13	
3	167	2.42	42.0	5.75	60.2	3.46	2.29	2.11	
4	168	2.69	30.0	6.05	56.8	3.43	2.62	2.28	
5	171	2.20	43.0	6.61	61.5	4.06	2.55	1.72	
6	172	2.32	44.0	6.95	53.8	3.74	3.21	1.72	
Average Values		2.39	40.7	6.13	59.1	3.62	2.51	1.99	
B. Rabbits Subjected to Tourniquet Shock									
1	141@	2.35@	74.8	4.18	52.1	2.18	2.00	1.66	17
2	146	2.41	60.0	4.89	49.7	2.43	2.46	1.38	35
3	147	2.64	47.5@@	4.31	58.3	2.51	1.80	1.91@@	9@@
4	166	2.47	80.0	4.96	46.1	2.29	2.67	1.59	30
5	173	2.14	64.0	4.77	51.7	2.47	2.30	1.23	29
6	175	2.19	74.0	4.67	56.4	2.63	2.04	1.33	24
Average Values		2.37	66.7	4.63	52.4	2.43	2.20	1.51	24
Percent Deviation from above Normal Values			+63.8	-24.4	-11.3	-32.9	-12.4	-24.1	

* Calculated from columns 5 and 6

** Calculated as difference between columns 5 and 7

*** Percent decrease of specific ABC values of 'shock' serum from the normal values determined in the same experiments

@ Sera of rabbits No. 141, 144, and 151 pooled (weights: 2.35, 2.28, and 2.41 kg respectively)

@@ The NPN content of this serum indicates a low degree of shock which might be responsible for the small decrease in the specific ABC value

Table 7 shows that certain differences were found between normal and "shocked" rabbits in the relative concentration of the various serum globulin components. Alpha$_1$ and alpha$_2$ globulin were elevated in the injured animals whereas the beta globulin components did not deviate markedly from normal. The average gamma globulin fraction was reduced in the "shocked" rabbits. Typical patterns are given in Figure 1.

TABLE 7

ELECTROPHORETIC ANALYSIS OF SERUM PROTEINS
OF NORMAL AND 'SHOCKED' RABBITS

Protein Components in Percent of Total Protein

Rabbits		Total Protein gm.%	Albumin	Alpha$_1$	Alpha$_2$	Beta$_1$	Beta$_2$	Beta$_3$	Gamma	u (Albumin) in 10^{-5} Cm^2 Sec^{-1} $Volt^{-1}$
Treatment	No.									
Normal	149	5.58	58.8	1.6	5.8	6.3	10.8	3.2	13.5	6.59
,,	153	5.84	63.2	2.6	8.5	4.8	10.1*	2.0	8.8	6.62
,,	167	5.75	60.2	2.8*	7.1	5.2	11.3	3.7	9.7	6.48
,,	168	6.05	56.8	2.5*	6.7	6.5*	9.5	3.0	15.0	7.00
,,	171	6.61	61.5	2.0	7.0	7.3	8.6	2.2	11.4	6.74
,,	172	6.95	53.8	1.2	8.5	12.0	9.6	2.7	12.2	6.57
Average		6.13	59.1	2.1	7.3	7.0	10.0	2.8	11.7	6.67
Average in gm. %			3.62	0.13	0.45	0.43	0.61	0.17	0.72	
Tourniquet Shock	141**	4.18	52.1	5.3*	14.6	9.0	11.7	2.7	4.6	6.68
,,	146	4.89	49.7	3.9*	12.6	9.3	10.0	3.7	10.8	6.76
,,	147	4.31	58.3	3.5*	7.8***	9.4*	10.2	3.3	7.5	6.94
,,	166	4.96	46.1	5.7*	18.2	10.5	10.1	3.6	5.8	7.00
,,	173	4.77	51.7	5.0	17.1	7.7	9.1	3.4	6.0	6.96
,,	175	4.67	56.4	9.3	9.8	10.3	6.8	2.6	4.8	6.88
Average		4.63	52.4	5.4	13.4	9.4	9.8	3.2	6.8	6.87
Average in gm %			2.43	0.25	0.62	0.43	0.45	0.15	0.30	

* Dividing line between this and the following component by interpolation (see Experimental Procedures).

** See Footnote @ in Table 6.

*** The NPN content of this serum indicated a low degree of shock which might be responsible for the 'normal' alpha$_2$ globulin value.

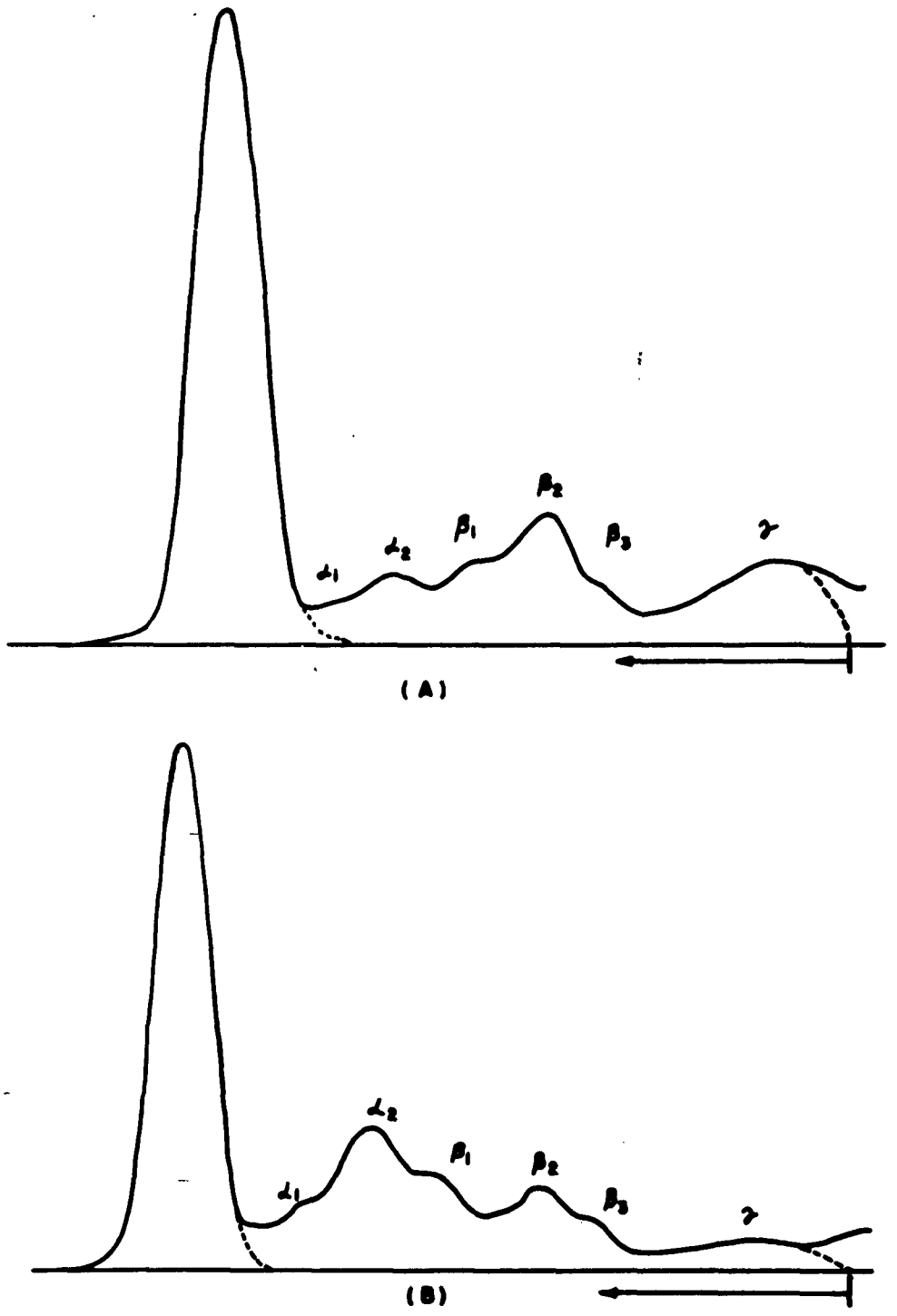

FIG. 1. TYPICAL ELECTROPHORETIC PATTERNS OF THE SERUM OF (A) A NORMAL RABBIT (NO. 149, TABLES 6 AND 7) AND (B) A "SHOCKED" RABBIT (NO. 173, TABLES 6 AND 7). DESCENDING BOUNDARIES. MICHAELIS BUFFER PH 8.6, μ = 0.1.

A fall in the albumin level and a decrease in the albumin/globulin ratio, as well as an increase in the alpha globulin component are well known to occur in the serum as a characteristic response to a variety of stressors (1). These abnormalities have not been observed, however, in rats subjected to tourniquet shock under comparable conditions (2). It is possible that the failure to demonstrate similar changes in the serum proteins of the shocked rats was due to hemoconcentration which has been found to occur in rats subjected to this form of traumatic shock (15). No significant hemoconcentration was observed in the "shocked" rabbits (Tables 4, 5).

The present observations on the changes in the serum protein composition of "shocked" rabbits may be considered complementary to the findings of Moore and co-workers. In studies on tourniquet shock in mice (16) and dogs (17), these authors gave direct evidence for a transfer of serum albumin into the traumatized tissue. They could not demonstrate a similar shift of alpha or beta globulins into the affected areas. The data of Table 6 indicate that the decrease of the total serum protein in the "shocked" rabbits was composed of a reduction of albumin as well as globulin in the approximate ratio 3:1. A recalculation of the average percentage composition of the globulins in gm% (Table 7) revealed that the lowering of the globulin level was caused mainly by a decrease of the gamma globulin and, to a lesser degree, the $beta_2$ globulin component.

The determination of the ABC is based on the proposition that azorubin is bound exclusively to the albumin component of the serum proteins. An electrophoretic analysis of a mixture of rabbit serum and azorubin at pH 8.6, 7.2, and 6.2 demonstrated this to be true. As could be seen (using color film) on the descending patterns, the dye was attached exclusively to albumin without the globulin components participating in the binding.

It has been pointed out in the Experimental Procedures and Table 2 that the specific ABC values of normal rabbit sera were found to be constant over a period of weeks. They did not show any marked variation from animal to animal. The specific ABC values obtained for the serum albumin of the rabbits subjected to tourniquet shock were 24% lower than those observed for albumin of normal serum (Table 6). This decrease is considered significant since the inherent (methodical) error of the chromatographic method (6), caused by differences in the albumin concentration of the sera, is less than 1% for the albumin concentrations of the present sera (average values 3.62g% and 2.43g% in the sera of normal and "shocked" rabbits,

respectively; see Figure 2 in reference (6)). In accordance with the mortality data (Table 3), one of the six injured rabbits (Table 6, B) did not show a marked elevation of the NPN value, indicating a less severe condition of shock. It seems noteworthy that in this case (Table 6, Experiment 3) the deviation of the specific ABC value from that of the normal control was much lower than in the other 5 experiments.

Table 8 shows the specific ABC of serum albumin from normal and "shocked" rabbits, calculated as moles azorubin per mole albumin, in comparison with the corresponding values for other species. All values were determined by the chromatographic procedure under similar conditions. The binding capacity of rabbit serum albumin was found to fall between those for human and rat albumin on the one side, and porcine, bovine and canine albumin on the other side.

TABLE 8

AZORUBIN-BINDING CAPACITY OF SERUM ALBUMIN OF VARIOUS SPECIES

Albumin	r Moles Azorubin per Mole Albumin*	Reference
Human Serum, Normal	3.02	19
Crystalline Form**, Human	2.70	7
Rat Serum, Normal	1.71	2
Rat Serum, Tourniquet Shock	1.25	2
Rat Serum, CCl_4 treatment	1.14	2
Rabbit Serum, Normal	1.37	Table 6
Rabbit Serum, Tourniquet Shock	1.04	Table 6
Purified Form, Porcine@@	0.95	--
Bovine Serum	0.96	6
Crystalline Form**, Bovine#	0.58	--
Purified Form, Canine@@	0.24	--

* Molecular weight of 69000 used for albumin of all species studied.

** 3.8 per cent solution in Ca^{++} and Mg^{++} free Krebs buffer.

@ 2.5 per cent solution in Ca^{++} and Mg^{++} free Krebs buffer.

@@ Fraction V, prepared from plasma by the cold alcohol fractionation procedure; purity approximately 85%. We are grateful to Dr. J. B. Leah, Biochemical Research, Armour and Company, Chicago, Illinois, for kindly supplying these preparations.

\# Armour and Company, Chicago, Illinois.

The biochemical mechanism underlying the decreased ABC values which were observed in the "shocked" rabbits as well as in other species under various conditions (2, 3) is not known. It is believed that a reduced ABC may be caused in vivo by metabolic alterations which result in the binding of abnormally high amounts of certain anions to serum albumin (18), in a similar way as a lowering of the ABC values of serum or serum albumin could be produced in vitro by the addition of small amounts of fatty acids and related anions (7). This problem will be discussed in a later report.

IV. SUMMARY

1. A procedure to produce tourniquet shock in rabbits was developed by occluding both fore legs. The method gave 87% mortality 24 hours after release of the tourniquet.

2. The absolute concentrations of total protein and albumin were found to be decreased markedly in the sera of rabbits subjected to tourniquet shock. The reduction in the serum albumin concentration was about three times as great as that of globulin. Non-protein nitrogen was elevated. Practically no hemoconcentration could be demonstrated.

3. The determination of the relative composition of the serum proteins by electrophoretic analysis showed a lowering of the albumin in the "shocked" animals. The alpha globulin components were increased. No marked change was observed in the relative beta globulin concentrations. The average gamma globulin values were reduced in shock.

4. The azorubin-binding capacity of the serum albumin was found to be decreased in the rabbits subjected to tourniquet shock.

V. RECOMMENDATIONS

Using the rabbit as an experimental animal, the mechanism of the lowering of the azorubin-binding capacity should be studied further. This is expected to result in a better understanding of metabolic alterations in shock as well as in other conditions causing abnormal ABC values.

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